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Machinations of a Maxwellian Demon

The mechanisms by which RNA polymerase moves along DNA during elongation have been difficult to determine experimentally. In this issue of *Cell*, Bar-Nahum et al. (2005) show that back and forth sliding of RNA polymerase on DNA may be coupled to bending of an α helix, which can alternately occlude and expose the NTP binding site. Transcription factors can regulate elongation by modulating this bending motion.

Boltzmann claimed an ambition to be the "Darwin of matter," to explain how fluctuations in ensembles of molecules could give rise to organized complexity over short timescales, much as variations within populations of organisms lead to ever-increasing complexity over geologic time (Prigogine and Stengers, 1984). He fell short of this ambition, but much of his program has been taken up by molecular biology, where the spontaneous creation of complexity is commonplace. Consider, for example, a transcription reaction: mix RNA polymerase, NTPs, and DNA in a pot and, within minutes, a macromolecule of high information content appears. How does this happen?

Recent structural studies of RNA polymerases reveal massive molecules with claw-like clamps that allow these molecules to hold onto DNA even as they move over thousands of base pairs (Zhang et al., 1999; Gnatt et al., 2001). At least two broadly distinct mechanisms have been proposed to explain how such movement may occur. In a power stroke mechanism, the energy of NTP hydrolysis is stored in a transient polymerase conformation that relaxes at the end of bond formation to propel the elongation complex by one nucleotide along the DNA. In a mechanism that has been variously dubbed "passive sliding," "translocational (or positional) equilibrium," or "Brownian ratchet," the clamps are presumed to be loose enough that the elongation complex can jiggle back and forth on the DNA in response to molecular collisions (Toulme et al., 1999; Bai et al., 2004). Paused or halted elongation complexes have, in fact, been shown to slide back and forth on the DNA, with their equilibrium positions determined by the relative stability of the competing RNA:DNA hybrids that form at different positions on the template (Nudler et al., 1997; Kommisarova and Kashlev, 1997; Palangat and Landick, 2001). This observation would tend to support the Brownian ratchet mechanism, but it is not at all clear that the mechanisms that determine the position of a halted elongation complex are relevant to an actively transcribing enzyme since the energy of a power stroke is expected to dissipate once the polymerase stops hydrolyzing NTPs. In fact, crystal structures of RNA polymerase have revealed a helix (the F helix) that can bend to occlude the NTP binding site in the polymerase (Figure 1). The NTP binding site is also the site that is occupied by the 3′ end of the RNA in the pretranslocated position, so the bending of the F helix could serve to push the polymerase from the pretranslocated to the posttranslocated state. Is the F helix therefore an actively driven piston that pushes the polymerase forward? Or is it, as Bar-Nahum et al. (2005) suggest, a Brownian pawl whose bending and straightening movements are driven by thermal fluctuations?

To explore this question, Bar-Nahum et al. (2005) isolated two mutants in the G loop, a region adjacent to the F helix that has been proposed to regulate F helix conformation. One of these (G1136S) creates a juggernaut polymerase that elongates more rapidly than wt and pauses and terminates less. The other (I1134V) has a complementary effect and results in slower elongation and more termination and pausing. Using exonucleases and crosslinkers whose points of attachment are sensitive to whether the F helix is bent or straight (Epshtein et al., 2002), Bar-Nahum et al. (2005) correlate these phenotypes with the effects of the mutations on halted elongation complexes. Thus, the I1134V mutation is found to enhance polymerase backtracking because it shifts the F helix equilibrium toward the bent state, in which it tends to disrupt the 3′ base pair (Figure 1), while G1136S increases the rate of transition between bent and straight conformations and in this way enhances forward translocation of the complex. The observation that the mutations' effects on transcription rates, pausing, and termination correlate with their effects on the positional equilibrium of halted complexes is perhaps the best evidence to date that translocation during elongation operates similarly to the passive sliding seen in halted complexes.

In a supplement, Bar-Nahum et al. (2005) mathematically model their mechanism to evaluate its predictive power and agreement with experiment. This is important because, despite the authors' insistence to the contrary, not all of their mechanism's implications are intuitive (at least not to me), so it is good to see that a mathematically explicit description accords with reality. For example, their modeling predicts that changes in the rate of transition and equilibrium between the F helix bent/straight conformations will affect fidelity because occlusion of the NTP binding site by the bent F helix reduces the effective NTP concentration at the active site. The prediction that emerges is that G1136S will misincorporate more than wt while I1134V will misincorporate less, and the misincorporation properties of G1136S and I1134V are indeed found to validate this expectation. It is not immediately clear, however, why such mechanisms are needed. For example, if the affinity of the active site for complementary and noncomplementary NTPs is appropriately set (relative to physiological NTP concentrations), fidelity can be achieved without an F helix. The answer may lie in the regulatory flexibility provided by this helix. A simpler mechanism could be optimized for fidelity and rapid elongation but only for a single NTP concentration. But NTP concentrations vary during the life of the cell, and, because of sequence effects on

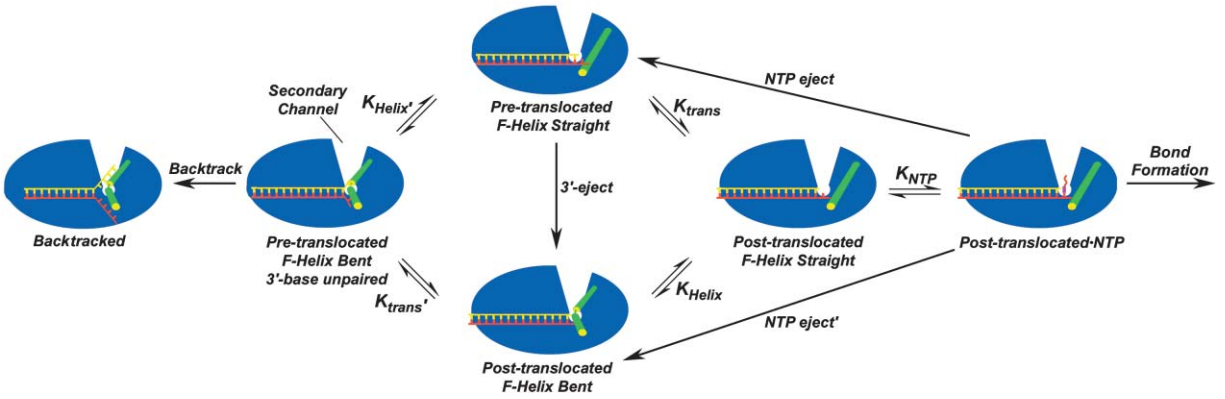

Figure 1. The Translocational Scheme Proposed by Bar-Nahum et al. (2005)

Different translocational and conformational states are in rapid equilibrium, but ejection steps are improbably reversible on the timescale of bond formation, while reversal of backtracking is slower than catalysis (RNA is yellow; DNA is red; F helix is green).

the positional equilibrium of the enzyme, the effective concentration of reactants in the active site varies over a wide range as the polymerase traverses the DNA. The authors therefore propose that elongation factors like NusA and NusG work by modulating the F helix bent/straight equilibrium to either enhance or mitigate the effects of pause and termination signals embedded in the DNA.

This picture of RNA polymerase mechanism would have pleased Boltzmann. The fashioning of RNA polymerase depended on random mutation and selection acting over eons. The machine that emerged from this evolutionary process has not forgotten its roots and now harnesses the sea of random thermal fluctuations in which it swims to generate organized complexity in the space of minutes.

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Antagonizing Wnt and FGF Receptors: an Enemy from within (the ER)

In this issue of *Cell*, Yamamoto et al. (2005) describe a novel molecule, Shisa, which functions in the endoplastic reticulum (ER) to prevent maturation of Frizzled (Fz) serpentine receptors and fibroblast growth factor receptor (FGFR). Shisa thus antagonizes Wnt and FGF signaling cell-autonomously, thereby promoting anterior patterning in *Xenopus*.

Regulation of growth factor signaling has central roles in development. Prominent examples include *Xenopus* dorsoventral and anterioposterior patterning by the Spemann-Mangold organizer, which modulates Wnt, bone morphogenetic protein (BMP), and Nodal signaling via a plethora of secreted antagonists (De Robertis and Kuroda, 2004). Particularly, the head-inducing activity of the organizer can be accounted for by combined actions of antagonists for Wnt (Dkk-1 and Frzb), BMP (Chordin and Noggin), and a multivalent antagonist for Wnt, BMP, and Nodal (Cerberus) (Niehrs, 2004). These extracellular molecules bind to the growth factor or the receptor to prevent ligand-receptor engagement (Semenov and He, 2003). Yamamoto et al. (2005) now reports a new molecule, Shisa, which regulates growth factor signaling in head development via a novel mechanism.

Shisa mRNA is expressed specifically in the organizer and its derivative, the anterior mesoderm/endomesoderm (where Shisa was isolated), which is the "head organizer" responsible for head induction (Niehrs, 2004); Shisa is also expressed in anterior neuroectoderm (Figure 1; Yamamoto et al., 2005), the tissue responsive to the head organizer. Shisa has homologs in zebrafish, mouse, and human; and Shisa protein can be secreted into conditioned medium in cell culture (Yamamoto et al., 2005). Shisa overexpression in *Xenopus* embryos expands the anterior structures and together with a BMP